

The Effect of Handwriting on Cortical Excitability:

A TMS study

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*I declare that this report is my own original work and that contributions of other have been
duly acknowledged.*

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Abstract

The current study investigated the effect of a handwriting task on cortical excitability in the primary motor cortex using transcranial magnetic stimulation (TMS). Seventeen participants (10 female) took part in a single session during which the amplitude of motor evoked potentials (MEPs) was measured in response to single and paired pulse TMS stimulation. Measurements were taken at baseline, immediately after a handwriting task and again 15 minutes after task completion. It was hypothesised that the handwriting task would cause a change to cortical excitability in the form of an increase in facilitation and a decrease in inhibition, as demonstrated by greater mean MEP amplitude and decreased short interval cortical inhibition (SICI) ratios, respectively. This study failed to detect a significant effect of handwriting on cortical excitability. Whether this is due to the absence of an overt effect, a methodological shortcoming associated with the exploratory nature of this study or a random fluctuation is unclear. The main implication of this study is that overlearned tasks such as handwriting represent a currently under-investigated area and further research would be of benefit for the TMS field.

A large proportion of the cognitive and motor behaviours in which we engage on a daily basis consist of overlearned tasks. These skills, such as walking, speaking, driving or handwriting were initially challenging when novel, but once acquired, can be carried out with minimal conscious effort (Willingham, 1998). Performance fluctuates very little as a function of practice (Ungerleider et al., 2002) and activities are often executed simultaneously or in conjunction with more cognitively demanding skills (Logan, 1979; Poldrack et al. 2005).

Given the extent to which we engage in overlearned tasks, surprisingly little is known about how these activities might induce or interact with neuroplastic changes to cortical excitability (CE) in the motor cortex. Although a great deal of transcranial magnetic stimulation (TMS) research focuses on motor learning and CE, the neural basis of overlearning everyday tasks and the effect of engaging in these tasks on CE is largely overlooked. This is in part due to an historical focus in research on the effect of learning novel or highly contrived tasks such as finger sequences or forced abductions (eg. Butefisch et al. 2000; Koenke et al. 2006; Stefan et al. 2006;).

Novel-task paradigms offer increased experimental control by eliminating potential effects of differential expertise, thus allowing causal inferences to be made about the relationship between CE and motor learning. As studying how people learn or overlearn everyday motor skills would require greater complexity in terms of research design, a blind spot seems to have evolved in terms of researching the effects of everyday tasks and CE more generally. While it seems likely that some of the observations associated with learning novel, abstracted tasks might also hold true for everyday tasks, it is also quite possible that generalisability of these findings to overlearned motor behaviour could be limited.

Decades of TMS research have robustly established that motor activity associated with novel tasks causes changes to CE in terms of patterns of excitation and inhibition (e.g.

Garry & Thomson, 2009; Karabanov et al., 2012; Pascual-Leone, Grafman & Hallett, 1994) but the extent to which similar changes are evoked by motor activity associated with overlearned tasks is currently unknown. If an overlearned task such as handwriting were to affect CE, this would be potentially problematic in terms of current TMS protocols, which generally treat participants arriving at laboratories as blank cortical slates and do not control for engagement in overlearned tasks prior to research participation. The potential concern in this practice is that cortical activity is continuous and CE is inherently dynamic, which, taken together means that participants already have a history of synaptic activity prior to engaging in any research. If overlearned tasks such as handwriting have the capacity to modulate CE in a similar way to novel motor tasks due to their shared physical demands, it stands to reason that overlearned tasks might also have the capacity to interact with the induction of subsequent neuroplasticity as novel or abstracted motor tasks have been observed to do (e.g. Goldsworthy et al. 2014; Iezzi et al., 2008; Rosenkranz, Kacar & Rothwell, 2007). If indeed overlearned tasks do have the capacity to modulate metaplastic mechanisms, it would be necessary to reconsider current TMS methodologies and potentially control for overlearned tasks.

Metaplasticity refers to activity-dependent mechanisms which regulate the expression of synaptic plasticity within neural networks (Abraham, 2008). In other words, metaplasticity describes processes that control the amount or direction of synaptic plasticity which can be induced by subsequent plasticity induction protocols after a given history of synaptic activation. Many metaplastic mechanisms appear to be guided by the principle of homeostasis, ensuring that neural networks maintain an adaptive level of dynamic flexibility by setting limits on the amount of long-term-potential (LTP) or long term depression (LTD) which can be induced by synaptic activity (Murakami et al. 2012).

Homeostatic metaplasticity is described by the Bienenstock-Cooper-Munro theory of bi-directional synaptic plasticity (Bienenstock et al. 1982), which maintains that synaptic plasticity is bi-directional (i.e. there is a possibility of evoking either LTP or LTD) and that the threshold for inducing either effect at any given time varies as a function of previous post-synaptic activity. According to this model, a history of high frequency activity causes an increase in the threshold for induction of LTP and a decrease in threshold for LTD induction, whereas low frequency synaptic activity will cause a reverse effect, allowing LTP to be induced at a lower threshold, and increasing the threshold for LTD. As such, metaplastic effects cannot be observed immediately after the activity that causes them, but rather, only when plasticity is subsequently induced.

The idea that overlearned tasks undertaken prior to research participation might cause overt changes to CE or influence subsequent plasticity induction seems plausible given that many of the tasks observed to cause changes to CE in a laboratory setting share some parameters with overlearned tasks (e.g. Byblow & Stinear, 2006; Garry, Kamen & Nordstrom 2004; Goldsworthy et al. 2014). A few rare studies on overlearned tasks and CE support this hypothesis, particularly when tasks have a linguistic component (Fililpovic et al. 2008; Lo & Fook-Chong, 2004; Papathanasiou et al. 2003).

The frequency with which humans engage in handwriting, an overlearned task with a linguistic and fine motor component is high, especially among students, a group likely to be heavily represented as participants in TMS research given the reliance on undergraduate student populations for research participation. Levels of response-variability observed in TMS research are also commonly high, and while this is likely to be related to the physiological complexity of the neural circuitry underpinning the modulation of CE, it may also be driven by participants' differential history of engagement in overlearned tasks such as handwriting.

TMS

Transcranial magnetic stimulation refers to a non-invasive technique by which an electrical field is created in neural tissue in response to magnetic pulses emitted by a wire coil placed on the outside of the scalp (Kobayashi & Pascual-Leone, 2003). This electrical field causes a depolarisation of neurons, and if stimulation is of sufficient intensity, the generation of action potentials. When TMS is applied to the motor cortex, excitation of pyramidal neurons in the corticospinal tract results in a volley of waves being conducted along the spinal cord causing motor evoked potentials (MEPs) that can be detected and measured in the corresponding anatomical area on the contralateral side of the body, providing an index of corticospinal excitability (CE) (Bashir et al. 2010). Corticospinal excitability refers to how responsive the corticospinal circuit is to stimulation at any given time. In other words, CE describes the relationship between the input and output of the circuit and is often assessed by placing electrodes on a target muscle in the hand, locating the representation of that muscle in the contralateral primary motor cortex (M1) and measuring the effects of stimulation on the output of the target muscle. Varying the pattern of TMS pulses in terms of timing and intensity can allow the characterisation of different components of the neural circuitry which comprise CE.

Response Variability

High levels of both inter- and intra-subject variability are commonly found in MEP amplitudes recorded in TMS research and despite considerable research into influential factors, the source of a large amount of this variability remains poorly understood (Bestmann & Krakauer 2015; Choudhury et al. 2011; Hamada et al. 2013). Some broad trends have emerged, such as a tendency towards decreased intra-subject variability at higher levels of TMS stimulation (Pitcher, Ogsden & Miles, 2003) and among subjects with a higher resting

motor threshold (Cuypers, Thijs & Meesen, 2004) or an increase in inter-subject variability among women and older adults (Pitcher, Ogsden & Miles 2003), but much variability remains unaccounted for.

A recent review of the uses and interpretations of MEPs by Bestmann and Krakauer (2015) emphasises the physiological complexity of MEPs elicited via TMS, noting that MEPs can be considered a summation of cortico-spinal, intra-cortical and trans-cortical contributions to excitability and that the respective influences of these elements (and therefore any potential modulators) can be difficult to quantify. MEPs have been observed to comprise a series of cortico-spinal volleys called direct waves (D-waves) and indirect waves (I-waves) which are characterised by different generators and latencies (Hamada et al. 2013). Di Lazzaro and Ziemann (2013) suggest that the characteristics of these MEP components are best described at the most basic level by a canonical microcircuit model of cortical input-output, featuring excitatory pyramidal neurons in layers II and III (P2 and P3) as well as the large, fast-conducting pyramidal tract neurons (PTNs) in layer V, and a network of inhibitory interneurons. According to this model, D-waves, which are the first volley of excitation to descend the spinal cord are generated by direct stimulation of the axons of PTNs in the white matter. Early I-waves (I1) are believed to be generated by mono-synaptic, excitatory inputs of P2 and P3 neurons onto PTNs as a result of axonal stimulation by the TMS pulse and later I-waves are generated by circuits involving reciprocal excitation between P2, P3 and PTNs as well as modulation by networks of inhibitory GABAergic interneurons.

Given that this is a complex circuit and that MEPs can be modulated by different components of this circuit, Bestmann and Krakauer (2015) emphasise that caution needs to be taken in interpreting the drivers of any changes on the basis of MEP amplitude alone. It also needs to be borne in mind that this is not a closed circuit, and there is evidence to support the idea that changes to MEPs elicited may be the result of afferent inputs from other areas,

potentially reflecting cognitive processes occurring elsewhere in the brain (e.g. Klein-Flügge & Bestmann 2012; Klein-Flügge et al. 2013). This may be particularly true of changes to MEPs in response to tasks featuring a linguistic component. For example, Papathanasiou et al (2003) observed increased CE during a visual search task involving a linguistic component in which participants were required to be physically inactive. Bilateral measurement of the first dorsal interosseous (FDI) muscles revealed larger MEPs in the right rather than the left hand, possibly associated with the predominant lateralisation of language to the left hemisphere. Their finding emphasises the potential of modulation of CE in the motor cortex via upstream changes in cortical activity associated with cognition. However, their study examined CE during task execution, so it remains unclear as to whether any changes to CE endured beyond task completion. If cognitively induced changes to CE of a linguistic origin were to persist post task, this might have profound implications for future TMS protocols as researchers would need to consider the possibility that baseline measurements of CE (such as resting motor threshold, which is commonly used to determine stimulus intensity for subsequent measures) might be affected by prior engagement in reading or writing (for example, consent forms or information sheets associated with research participation, or indeed activities undertaken prior to participation).

Another related factor influencing response variability is that due to the dynamic nature of neural circuits, patterns of excitation and inhibition are liable to change depending on a participant's history of synaptic activation (Ridding & Ziemann, 2010). This means that the same experimental protocols can evoke different responses at different times within a single participant (eg. Rosenkranz, Kacar & Rothwell, 2007), and that differences observed between multiple participants might have the potential to reflect the influence of extraneous variables relating to synaptic activity prior to testing rather than the independent variable (Goldworthy et al. 2014)).

Metaplasticity

Homeostatic metaplasticity at a systems level has been observed in the human primary motor cortex, as demonstrated by changes in CE measured by MEPs (eg. Fricke et al.; 2011; Goldsworthy et al. 2014; Murakami et al. 2012). These metaplastic effects have been found in both inhibitory and excitatory circuits by pairing different combinations of continuous and intermittent transcranial magnetic theta burst stimulation (cTBS and iTBS) and comparing their respective input-output curves (IO) using TMS to evoke MEPs and calculate IO of SICI (Murakami et al., 2012).

In excitatory corticospinal circuits Murakami et al. observed predictable baseline IO responses following non-primed iTBS and cTBS (increased and decreased IO of MEPs, respectively) and a homeostatic metaplastic effect when pairing identical protocols as demonstrated by a decrease in the magnitude of plasticity induced by the second protocol of each pair relative to baseline and pairing of non-identical protocols. The authors also found evidence of homeostatic metaplasticity in inhibitory circuits, with decreases in IO of SICI observed following paired excitatory protocols and increases in IO of SICI after paired inhibitory protocol. Measuring metaplastic effects by comparing different combinations of plasticity-inducing stimulation protocols in the motor cortex provides something of an analogue for naturally occurring LTP and LTD-like processes and homeostatic metaplastic effects can also be observed in response to or in interaction with motor activity (eg. Jung & Ziemann, 2009; Stefan et al. 2006).

Additionally, there is considerable evidence of interactions between or modulations of different stimulation protocols or stimulation protocols and motor activity of a non-homeostatic nature. For example, Iezzi et al (2008) found that the introduction of a phasic finger movement task caused a reversal in polarity of plasticity subsequently induced (rather

than just a suppression of the extent of facilitation and inhibition). Other studies, such as Rosenkranz, Kacar and Rothwell (2007) have observed changes in the interaction between motor activity and plasticity induction depending on the phase of motor learning, with a reversal in polarity of response to paired-associative-stimulation (PAS). When applied to cortex which has not been primed, PAS with a 25ms inter-stimulus interval (PAS25) produces an LTP-like effect, however, Rosenkranz found PAS25 following motor activity led to an LTD-like effect during early stages of motor learning which was not observed following motor activity in later stages of motor learning.

Studies looking at metaplastic effects in the motor cortex evidently vary profoundly in terms of both the protocols used and also the nature of the relationships observed. Inter-subject response variability to plasticity induction protocols within studies in this field is also often quite high (eg. Hamada et al. 2013; Stefan et al. 2006). A recent study by Goldsworthy et al. (2014) suggested that prior activation of target hand muscles may lead to increased inter-subject variability following plasticity induction. This finding has implications for the interpretation of studies looking at metaplastic effects because increases in variability can be understood as a decrease in net effect when analysed on a group level, which may be interpreted as a metaplastic suppression of LTP or LTD-like changes. This also has implications for methodologies by future studies in this field that use TMS to measure changes in CE because tonic contractions such as those used by Goldsworthy et al. are frequently employed to establish active motor threshold in order to adjust stimulus intensity, or to aid the location of the cortical representation of a target hand area. Finally, this finding highlights the necessity of controlling for participants' activity prior to participating in TMS studies.

SICI

Short latency or short-interval intra-cortical inhibition (SICI) is a widely used TMS technique which measures levels of inhibition within the cortex by networks of inhibitory interneurons via a paired-pulse paradigm consisting of an initial conditioning pulse followed by a test pulse (Bashir et al. 2010). The conditioning pulse activates the circuit of inhibitory interneurons and the test pulse, delivered 1-6ms later, evokes an MEP which is measured and when compared to single-pulse MEPs, can provide an index of the inhibitory effect of the interneuron circuit (Di Lazzaro et al. 1998). These inhibitory circuits are maximally activated at levels of stimulation below the resting motor threshold (RMT), so the conditioning pulse is usually set at between 60-80% of RMT, with a supra-threshold test pulse following. Resting motor threshold refers to the level of stimulation at which an MEP can be evoked from an individual when muscles are at rest. This is usually determined as the intensity at which an MEP with an amplitude >50mV can be measured in 50% of trials. Cortical inhibition as measured by SICI has been observed to affect later components of the test MEPs (indirect waves caused by synaptic facilitation) rather than the early components caused by direct stimulation of the axons of pyramidal tract neurons by the TMS pulse itself (direct waves) (Di Lazzaro et al. 1998). Studies using pharmacological adjuncts have observed these inhibitory circuits to be GABAergic, most likely mediated by GABA-a (Rothwell et al. 2009).

Decreases in inhibition are often observed to accompany neurological conditions in a clinical setting but are also associated with motor learning in a research context (Rothwell et al. 2009). In a motor learning context, disinhibition is thought to play a role in long term potentiation (LTP) in M1 by facilitating neuroplastic change at a synaptic level, as well as potentially unmasking existing excitatory inputs onto pyramidal tract neurons (Pascual-Leone, Grafman & Hallet, 1994). A review of the literature on overlearned tasks and cortical

excitability failed to locate any studies which measured changes to SICI in response to engaging in an overlearned task. However, a study by Filipovic et al. (2008) measured cortical silent period (SP) (another index of cortical inhibition) and observed an effect of disinhibition during a handwriting task which was interpreted as being associated with the linguistic demands of the task.

Silent period refers to the phenomenon by which electromyographic (EMG) activity associated with the constant voluntary contraction of a muscle ceases for a few hundred milliseconds following the delivery of a TMS pulse above resting motor threshold (Bashir et al. 2010). The silent period is usually measured from the end of the MEP evoked by the TMS pulse until the point at which EMG activity caused by the continued contraction resumes. Both spinal and cortical inhibitory mechanisms are believed to contribute to different stages of SP, with inhibitory spinal mechanisms affecting the earlier, direct waves and cortical networks of inhibitory interneurons affecting the later, indirect waves (Chen, Lozano & Ashby, 1999).

Evidence of disinhibition from studies looking at SP during linguistic tasks (Filipovic et al. 2008; Lo & Fook-Chong, 2004, Papathanasiou et al., 2004), along with the decreases in SICI observed in response to fine motor tasks which feature similar movements to handwriting and rely on the same effectors (eg. Garry, Kamen & Nordstrom, 2004) makes it seem plausible that handwriting might cause a decrease in SICI and given the lack of literature on this subject, worthy of investigation.

Overlearned tasks

Overlearned motor skills consist of movement sequences which are largely automated, can be conducted with minimal conscious effort and are not vulnerable to decreases in proficiency if not practiced for long periods of time (Doyon, Penhune & Ungerleider, 2003). Most research

looking at changes in CE associated with motor learning focuses on novel tasks, usually over a short period of time, either within a single session or over a period of a week or two (eg. Bütetfisch et al., 2000; Pascual-Leone et al. 1994; Koenke et al., 2006). Observations of disinhibition or increased MEP amplitude immediately following learning within a single session are common when comparing post-task to baseline levels (eg. Garry, Kamen & Nordstrom, 2004; Ziemann et al. 2001; Bütetfisch et al., 2000). There is also some evidence to suggest that patterns of excitation and activation change over subsequent sessions as proficiency increases, for example, Pascual-Leone, Grafman and Hallett (1994) found that areas in M1 representing muscles involved in a finger sequencing task increased in size and showed increased CE associated with behavioural gains during the learning process. These cortical changes abated as participants' knowledge of the sequence became automated. The time course of these changes led the authors to suggest that changes observed during the learning phase were consistent with the notion of unmasking existing connections and increasing synaptic efficacy associated with LTP and that the flexibility surrounding cortical modulation during learning could lead to structural changes in intra-cortical and subcortical networks as skills become overlearned.

Similarly, evidence from imaging studies show behavioural changes correlate with patterns of activation as motor learning occurs over time (e.g. Penhune & Doyon, 2002; Puttemans, Wenderoth & Swinnen, 2005). In their 2002 review of studies using neural imaging to investigate motor skill learning, Ungerleider, Doyon and Karni propose that early stages of learning involve rapid, dynamic increases in activity in cortical frontal lobe areas, the striatum and the cerebellum with some involvement of the primary motor area, but that over a period of weeks this gives rise to a slower re-organisation of the primary motor area, leading the authors to suggest imaging data are consistent with the hypothesis that

overlearning motor skills involves the recruitment of additional neurons in M1 to overlapping sequence-specific local networks within representation areas.

The idea of longer-term motor learning being underpinned by structural and functional changes in M1 is also supported by neurophysiological evidence taken from animal studies such as Rioult-Pedotti et al. (1998), who observed developments in horizontal networks of pyramidal cells in layers II/III of the primary motor cortex of rats after five days of practicing a skilled reaching task. Rioult-Pedotti et al. reported increases in amplitude of local field potential recordings and decreased propensity for LTP induction *in vitro* in the affected cortical areas, which was interpreted as indicative of LTP having occurred and increased the threshold for the induction of subsequent LTP. Increased synaptogenesis in layer V accompanied by increased size of representational area has also been observed in a similar paradigm by Kleim et al (2002), suggesting a clear association between behaviour, cortical excitability and changes to cell morphology in M1 which would be consistent with LTP.

There seems to be considerable evidence showing that the process of becoming proficient at a motor skill is associated with changes to M1 as learning occurs and also more enduring changes which persist past once task execution has ceased. In other words, the level of proficiency attained during learning is associated with differential patterns of cortical activation, cortical excitability and morphological changes in the motor cortex. The presence of these effects in studies of novel tasks further underscores the apparent lack of research conducted into the neurophysiological substrate of overlearned tasks we engage in on a daily basis, such as writing (Filipovic et al. 2008).

The very basic, abstracted, novel tasks commonly used to observe learning from a neurophysiological point of view are appealing because they allow for an increased level of

experimental control; findings are not confounded by differential levels of expertise and lengthy periods of skill development are not necessary for high levels of proficiency to be achieved. However, this means that very little is known about the effect of engaging in everyday motor tasks on the motor cortex, or how the development of new skills might interact with those already attained. One rare study, by Balas et al. (2007) looked at how engaging in an overlearned writing task (as compared to a writing task in an unfamiliar alphabet) could interfere with consolidation when learning a novel finger opposition sequence. The authors found a significant interference effect of the overlearned writing task on offline-gains (improvements in performance occurring between practice sessions) when participants were tested 24 hours later, which was not observed in the unfamiliar writing task condition or the control group. This is an interesting result insofar as dominant explanations of interference suggest that it is likely to occur when tasks are similar, making task parameters set in the first task vulnerable to supersession by those set in the second task (Shadmehr & Holcomb, 1997).

The findings of Balas et al. (2007) led the authors to suggest that practicing sequences of movement with very different attributes can nonetheless lead to interference if the tasks share a cortical representation area and potentially recruit neurons to specific local networks from the same pool, which would be a possibility in this scenario given that representation areas in M1 have been observed to be involved in executing novel tasks as well as coding for well-learned sequences (Ungerleider et al., 2002). On a practical level, Balas et al. (2007) suggested that these findings should be taken into account in therapeutic or experimental context in order to avoid potential disruption of motor learning.

Rationale, Aims and Hypotheses of the current study

There is currently a dearth of literature examining the effects of overlearned tasks such as handwriting on CE. There is some evidence of changes to CE during the execution of a handwriting task (Filipovic et al. 2008), but to date, we have been unable to find any investigation of the potential for handwriting to induce changes to CE which endure beyond task completion. Given that handwriting has been observed to interact with motor learning (Balas et al. 2007) and also features fine motor movements which have been observed to induce longer lasting changes to CE (Garry, Kamen & Nordstrom, 2004), it seems plausible that handwriting might cause a neuroplastic modulation of CE.

As TMS protocols rarely control for activities undertaken prior to research participation, there is a distinct possibility that if there was an effect of handwriting, this might influence subsequent measurements of changes in CE unbeknownst to researchers. This is likely to be especially relevant and worthy of further investigation given that a high proportion of participants are students and may frequently engage in handwriting prior to research participation. Also, because TMS research often reveals high levels of response variability and recent evidence suggests that prior motor activity may increase variability following plasticity induction (Goldsworthy et al. 2014), there would be major methodological implications if an effect of handwriting were to emerge.

The present study aims to address the current lack of literature on overlearned tasks and CE by measuring the effect of a short handwriting task on MEP amplitude and SICL. This investigation is exploratory in nature and will have the capacity to measure only an overt change, not any priming or metaplastic effects. It is hypothesised that the handwriting task will cause a significant increase in excitability in the primary motor cortex, as evidenced by increased amplitude of MEPs in response to single pulse TMS, and that there will be

significant effect of disinhibition, shown by a reduction in the difference between single and paired pulse MEPs.

Method

Participants

Seventeen participants (10 female) were recruited through advertisements at the University of Tasmania as part of a larger study after an initial calculation indicated that a sample of 20 participants, would be sufficient to detect a moderate effect size of $d = .66$ as calculated by G*Power 3 (Faul, Erdfelder, Lang & Buchner, 2007). Prospective participants underwent a brief medical screening and those with contraindications for TMS were excluded (see Appendix D for contraindications). Participants were aged between eighteen and forty-five years in order to be able to give consent and to exclude potential confounds due to changes in cortical excitability associated with increased age (Fujiyama et al. 2012). The mean age was 31.06 years ($SD = 6.54$). Participants were all right-handed to avoid variability associated with cerebral dominance among the left-handed (Isaacs et al., 2006). Course credit was offered to eligible students, and all participants were put in to a draw to win gift vouchers.

Apparatus and Materials

Two Magstim 200² stimulators (Magstim Co., Whitland, UK) were used to apply TMS through a single figure of eight coil attached to a BiStim module. Ag/AgCl electrodes were used to record electromyographic activity from the first dorsal interosseous (FDI) and the abductor pollicis brevis (APB) muscles. Recordings were amplified and band-pass filtered using CED1902 amplifiers (Cambridge Electronics Design, Cambridge, UK) before being sampled with a CED Power1401 data acquisition system and sweeps were collected using

Signal 4.0 software (Cambridge Electronics Design, Cambridge, UK). Participants completed the writing task using a ball point pen and paper and a text exemplar of 300 words printed on an A4 sheet in 12 point Times New Roman (See Appendix F).

Procedure

The following procedures were approved by the Tasmanian Social Sciences Human Research Ethics Committee (See appendix A). Participants underwent a short medical screening (see Appendix D) , were briefed as to the procedure and informed consent was obtained (see Appendix C for consent form). Participants were seated, the skin above the FDI and APB muscles was abraded to prevent impedance and electrodes were attached in a belly-tendon montage. The area of left motor cortex at which maximal MEPs could be obtained from the right FDI by stimulating moderately above threshold was located by moving the coil around the M1 hand area in small steps until maximal MEPs were consistently evoked. The coil was then placed at an angle with the handle at 45° from the midline and facing backwards to induce currents in a posterior-to-anterior direction across the central sulcus and the position of the coil was marked on participants' scalps using a felt-tipped marker to ensure a consistent coil position across trials.

Resting Motor Threshold (RMT) was established by beginning stimulation in this location at a suprathreshold intensity and then reducing intensity in increments of 2% of the maximum of the simulator output until no MEPs could be elicited over five consecutive pulses. Stimulation intensity was then increased in steps of 1% and the lowest intensity at which MEPS (with an amplitude of at least 50 μ V) were recorded in three of five consecutive pulses was considered the RMT (Garry et al. 2009). Cortical excitability was measured via short interval cortical inhibition (SICI); silent period (SP) and MEP recruitment curves four

times. Two measurements were taken at baseline, prior to the writing task, another immediately afterwards (post 0) and finally at 15 minutes post-task (post 15).

SICI was measured with ten paired pulses at each stimulation intensity. A conditioning pulse of 70% and test pulses at intensities between 120-140% of RMT (in 10% steps) with an interstimulus interval of 3ms. Ten single TMS pulses were also delivered at each intensity to obtain an MEP recruitment curve. Silent period was measured during a voluntary contraction of FDI and APB (at approximately 10% of maximal force) with 20 single pulses per measurement interval at a stimulus intensity of 130% of RMT. The measurement of silent period as well as the administration of single and paired pulses was conducted in a randomised sequence to avoid any order effects.

Design and Data Analysis

The current study used a within-subjects, repeated-measures design to examine the effect of the independent variable of handwriting on the dependent variable of cortical excitability. Cortical excitability was operationalised as mean peak-to-peak MEP amplitude in microvolts (mV) and SICI, which was measured as the ratio of the conditioned MEP (paired pulse stimulation) to the test MEP (single pulse stimulation). Measurements were taken just prior to; immediately following and at 15 minutes after completing a hand-writing task. Two sets of baseline measurements were taken for each participant in order to increase the reliability of estimates of SICI and MEP amplitude at rest. This was not feasible for post-task measurements given the time taken to administer two sequences of pulses immediately post-task would not have allowed for measurements to be taken at 15 minutes post-task.

Data for one participant was excluded on the grounds of high levels of background EMG activity making it difficult to distinguish MEPs. Additionally, silent period data was

discarded after it was established that in a large proportion of sweeps, there was insufficient evidence of background muscle activity to be able to discern the end of the silent period.

Single and paired-pulse MEPs were analysed using two separate repeated-measures ANOVAs for each muscle. SICI was analysed using a 3 (time: mean baseline, post 0 and post 15 minutes) x 3 (intensity: 120%, 130% and 140% of resting motor threshold) way ANOVA. MEP was analysed using a 3 (time: mean baseline, post 0, post 15 minutes) x 2 (tms type: test or conditioned) x 3 (intensity: 120%, 130% and 140% of resting motor threshold) way ANOVA. Both significant and non-significant main effects are reported, as are significant interaction effects. Multivariate tests were used, so sphericity is not reported. Because SICI ratios were not normally distributed, SICI was transformed to log SICI prior to analysis.

Results

MEP amplitude

As would be expected, mean amplitude of MEPs recorded at both the FDI and APB muscles was greater in the single pulse condition compared with the paired pulse condition, and increased at higher levels of stimulation intensity. In the FDI muscle, mean MEP amplitude in both the single and paired pulse conditions was greater 15 minutes following the writing task than measurements at baseline and immediately after the writing task; however, MEP amplitude immediately following the writing task was lower than at baseline in both TMS condition (see Table 1 for means and standard deviations). In the APB muscle, mean MEP amplitude was lowest at baseline and increased with each measurement in both the paired and single pulse conditions (see Table 2 for means and standard deviations). Significant main effects of TMS intensity and pulse type on MEP amplitude were observed for both muscles.

Table 1

Means and standard deviations of MEPs recorded from the FDI muscle across different conditions. .

Intensity	Conditioned			Test		
	Baseline	Post 0	Post 15	Baseline	Post0	Post 15
120%	0.55	0.68	0.58	1.53	1.49	1.65
	(0.58)	(1.13)	(0.53)	(1.43)	(1.20)	(1.41)
130%	1.24	0.81	1.17	2.47	2.08	2.38
	(1.47)	(0.77)	(1.41)	(2.37)	(1.67)	(1.71)
140%	1.60	1.64	2.06	2.79	2.90	3.47
	(1.55)	(1.68)	(2.00)	(2.22)	(2.32)	(2.62)

Note: Standard deviations are presented in parentheses. Stimulation Intensity is expressed as a percentage of RMT and all MEPs are in millivolts.

In the FDI muscle, the main effect of TMS intensity was highly significant, $F(2,14) = 11.69$, $p = .001$, $\eta^2 = .625$, as was the main effect of TMS type (conditioned vs test pulse), $F(1, 15) = 23.88$, $p = >.001$, $\eta^2 = .614$. There was also a main effect of time which was approaching significance, $F(2, 14) = 3.51$, $p = .058$, $\eta^2 = .334$ and a significant interaction effect between TMS type * TMS intensity, $F(2, 14) = 5.22$, $p = .02$, $\eta^2 = .427$. None of the other interaction terms were significant. Multivariate data can be found in Appendix G

Bonferroni corrected pairwise-comparisons showed that the three levels of stimulation intensity were all significantly different from each other and that there was a significant difference between MEP amplitude measured immediately following the writing task and 15

minutes afterwards but that neither of the post task measurements differed significantly from mean baseline (See Table 3).

Table 2

Means and standard deviations of MEPs recorded from the APB muscle across different conditions.

Intensity	Conditioned			Test		
	Baseline	Post 0	Post 15	Baseline	Post 0	Post 15
120%	0.85	1.04	1.04	1.72	1.93	2.29
	(0.91)	(1.21)	(1.11)	(1.31)	(1.55)	(1.90)
130%	1.60	1.72	1.72	2.83	2.78	3.04
	(1.33)	(1.90)	(1.84)	(2.37)	(2.03)	(2.20)
140%	2.12	2.20	2.80	3.36	3.41	3.99
	(1.71)	(1.92)	(2.14)	(2.26)	(2.00)	(2.12)

Note: Standard deviations are presented in parentheses. Stimulation Intensity is expressed as a percentage of RMT and all MEPs are in millivolts.

Analysis of MEP amplitude in the APB muscle yielded a similar picture; there were significant main effects of TMS intensity, $F(2, 14) = 13.3$, $p = .001$, $\eta^2 = .655$ and TMS type $F(1, 15) = 15.96$, $p = .001$, $\eta^2 = .515$, with a main effect of time which could be described as approaching significance $F(2, 12) = 3.13$, $p = .075$, $\eta^2 = .309$, however, no significant interactions were observed (see Appendix G for multivariate data). Pairwise

comparisons with a Bonferroni correction suggested that there were no significant differences in amplitude between measurements taken at baseline, following task completion or 15 minutes later. Once again, significant differences were observed in all comparisons of the three stimulation intensities (see Table 4).

Table 3.

Pairwise comparisons of MEP amplitude recorded at 3 different intensities at 3 time points at the FDI muscle.

factor	Comparison	<i>p</i>	Standard Error	95% CI for the difference between means	
				Lower limit	Upper Limit
Time	Baseline-Post0	1.00	0.14	-0.27	0.47
	Baseline-Post15	.604	0.14	-0.57	0.19
	Post0-Post15	.047*	0.10	-0.57	0.004
Intensity	120-130%	.001*	0.13	-0.95	-0.26
	120-140%	.001*	0.28	-2.08	-0.58
	130-140%	.005*	0.19	-1.23	-0.21

Note: P values have been Bonferroni corrected. Asterisks indicate significant *p* values.

Table 4.

Pairwise comparisons of MEP amplitude recorded at 3 different intensities at 3 time points at the APB muscle.

Factor	Comparison	<i>p</i>	Standard Error	95% CI for the difference between means	
				Lower limit	Upper Limit
Time	Baseline-Post0	1.00	0.25	-0.77	0.56
	Baseline-Post15	.174	0.20	-0.93	0.13
	Post0-Post15	.292	0.25	-0.76	0.16
Intensity	120-130%	.004*	0.20	-1.35	-0.26
	120-140%	.000*	0.28	-2.27	-0.74
	130-140%	.010*	0.20	-1.25	-0.16

Note: *p* values have been Bonferroni corrected. Asterisks denote significant *p* values.

SICI ratios

Mean Log SICI ratios of MEPs recorded from the FDI muscle showed a pattern of decreasing inhibition as intensity increased, and an increase in inhibition over time, with maximum inhibition observed 15 minutes after task completion (see Table 5). However, the 3x3 way ANOVA revealed non-significant main effects of intensity, $F(2,14) = 3.49$, $p = .059$, $\eta^2 = .33$, and time, $F(2,14) = .60$, $p = .56$, $\eta^2 = .08$, although intensity could be construed as approaching significance. The interaction term for the FDI muscle was also non-significant.

Multivariate data can be found in Appendix G. Bonferroni corrected pairwise comparisons revealed significant differences in effect of intensity on inhibition in stimulation at 120% versus 140%, of resting motor threshold, and but no significant differences between 120% and 130% or 130% compared with 140% (see Table 6). No significant differences were observed between any of the pairwise comparisons of the different levels of time.

Table 5

Mean Log SICI for FDI across different times and intensities.

Stimulation intensity	Mean baseline	Post 0 minutes	Post 15 minutes
120%	-0.42 (0.28)	-0.45 (0.41)	-0.47 (0.29)
130%	-0.39 (0.34)	-0.43 (0.32)	-0.42 (0.35)
140%	-0.29 (0.27)	-0.31 (0.21)	-0.32 (0.22)

Note: Standard deviations are presented in parentheses. Stimulation Intensity is expressed as a percentage of RMT

Table 6

Pairwise comparisons of log SICI ratios of the FDI muscle

				95% CI for the difference	
				between means	
-	Factor	Comparison	<i>p</i>	Standard Error	Lower limit Upper Limit
Time		Baseline-Post0	1	0.03	-0.52 0.11
		Baseline-Post15	.97	0.03	-0.53 0.12
		Post0-Post15	1	0.03	-0.76 0.08
Intensity		120-130%	1	0.03	-1.19 0.06
		120-140%	.05*	0.05	-2.85 0.00
		130-140%	.07	0.05	-2.33 -0.01

Note: *p* values have been Bonferroni corrected. Asterisks denote significant *p* values.

At the APB muscle, SICI ratios showed a similar pattern to the FDI muscle, in terms of decreased mean Log SICI at higher stimulation intensities, indicating lower levels of inhibition at higher intensities but in contrast to the FDI, inhibition at the APB muscle decreased over time (see table 7),.

The 3x3 ANOVA showed these trends were not indicative of a significant main effect of intensity ($F(2, 14) = 1.84, p = .57, \eta^2 = .208$) or a significant main effect of time ($F(2, 14) = 1.26, p = .31, \eta^2 = .153$) (see Appendix G). Once again, the time*intensity interaction was also found to be non-significant. Bonferroni corrected pairwise comparisons found no significant differences between any levels of the two factors (see Table 8).

Table 7

Means and standard deviations of log SICI across time and intensity in APB.

Stimulation intensity	Mean baseline	Post 0 minutes	Post 15 minutes
120%	-0.40 (0.38)	-0.34 (0.38)	-0.45 (0.41)
130%	-0.30 (0.34)	-0.30 (0.30)	-0.35 (0.37)
140%	-0.28 (0.23)	-0.29 (0.30)	-0.27 (0.31)

Note: Standard deviations are presented in parentheses. Stimulation Intensity is expressed as a percentage of RMT

Table 8

Pairwise comparisons of log SICI ratios of the APB muscle

				95% CI for the difference between means	
-	-	-	-	-	-
- Factor	Comparison	<i>p</i>	Standard Error	Lower limit	Upper Limit
Time	Baseline-Post0	1	0.03	-0.11	0.06
	Baseline-Post15	1	0.05	-0.10	0.15
	Post0-Post15	.472	0.03	-0.04	0.14
Intensity	120-130%	.249	0.04	- 0.20	0.04
	120-140%	.366	0.07	-0.30	0.07
	130-140%	1	0.06	-0.19	0.120

Note: *p* values have been Bonferroni corrected. Asterisks denote significant *p* values

Variability

The co-efficient of variation (CV) was calculated for each condition by dividing the standard deviation (SD) of the group at each intensity, time point and tms type by its respective mean. Dispersion of MEP amplitudes around the group mean was generally quite high, with means and SDs often having a similar value. Higher mean CVs were observed in paired pulse trials compared with single pulse trials, and an inverse relationship between CVs and stimulation intensity was observed (see table 9).

Table 9

Mean co-efficient of variation across the different conditions from the APB and FDI muscle.

Intensity	Conditioned			Test		
	Baseline	Post 0	Post 15	Baseline	Post0	Post 15
120%						
FDI	1.05	1.67	0.92	0.93	0.81	0.86
APB	1.08	1.17	1.06	0.76	0.81	0.83
130%						
FDI	1.19	0.95	1.21	0.96	0.81	0.72
APB	0.84	1.10	1.10	0.84	0.73	0.72
140%						
FDI	0.97	1.03	0.97	0.80	0.80	0.75
APB	0.81	0.87	0.76	0.67	0.59	0.53

Note: Intensities represent % of resting motor threshold

Discussion

This study aimed to explore the possibility that engaging in a handwriting task might cause an overt change to cortical excitability. It was hypothesised that a change in CE would be induced, and that this would manifest as increased MEP amplitude and a decrease in SICI ratios measured at both the FDI and APB muscles. None of these hypotheses were supported by significant main effects or interactions. Although mean amplitude of both single and paired pulse MEPs showed cortical excitability was at its highest 15 minutes after completing the writing task at both the FDI and APB muscles, the absence of a significant main effect of time at the designated alpha level means this observation cannot be justifiably interpreted as the result of the handwriting task rather than chance fluctuations. Similarly, no causal inferences can be made on the basis of the results of the analysis of SICI in relation to time as there were neither any significant main effects, nor clear trends apparent in the pattern of mean SICI ratios of MEPs from the two muscles.

The significant main effects of TMS intensity and TMS type on MEP amplitude which were observed were expected given that previous research has fairly robustly established the correlation between input intensity and output amplitude in the corticospinal circuit (e.g. Choudhury et al. 2011; Darling, Wolf & Butler, 2006; Pitcher, Ogston & Marsh, 2003;) as well as the inhibitory effect of paired pulse stimulation on MEP amplitude (e.g. Rothwell, Thompson & Kujirai 2009). However, these effects can be considered ancillary as neither relate directly to the hypotheses. As the current study failed to detect an overt effect of handwriting on CE at the determined significance level, the finding can be considered a null result. Because this study was exploratory by nature and the hypotheses were not supported by the data, this result cannot be interpreted with any degree of certainty.

It is entirely plausible that the current study failed to detect an effect because there was no effect to be found. In other words, handwriting does not have any influence on CE. While engaging in novel fine motor tasks (Garry, Kamen & Nordstrom, 2004) or abstracted thumb abduction tasks (Bueteifisch et al., 2000) causes enduring changes to CE, it is possible that despite having similar physical demands, handwriting tasks do not have a similar effect due to some other difference between these tasks and handwriting. One possibility is that changes to CE observed in motor learning could be a reflection of task novelty rather than the physical requirements of the task. Perhaps the changes to CE observed following novel tasks represent a neural flexibility which allows for novel task parameters to be more easily encoded. This would be adaptive in the early stages of learning, but might undermine the stability of automated skills if it were to continue to be induced simply by motor movement.

The idea that changed CE itself might be indicative of a change in flexibility which facilitates motor learning is conditionally supported by some studies reporting enhanced motor performance following the induction of plasticity by non-invasive brain stimulation techniques (Takeuchi & Izumi, 2015). Depending on the timing of plasticity induction and motor learning, Takeuchi & Izumi suggest that inducing changes in CE can either have a homeostatic effect (induction of an increase or a decrease in CE causes an effect of the opposite direction on learning) or a synergic effect (induction of a change in CE causes an effect of the same direction on learning). Synergic effects are more likely to occur when plasticity induction and motor learning are simultaneous or separated by a short period of time. Synergic effects are consistent with the idea that CE might index neural flexibility or associated with novel motor learning because of the directional association between CE and learning of novel tasks (e.g. Teo et al., 2011).

The finding of increased size and CE of representation areas in M1 associated with early motor learning by Pascual-Leone, Grafman and Hallett (1994) offers strong evidence for a

qualitative change in the neural production of motor behaviour associated with learning. The authors observed these changes only during the early learning phase of a novel motor task, and as the task became overlearned, these changes subsided. This would be highly consistent with an interpretation of the current results that suggests that there was no effect of handwriting on CE because overlearned tasks do not in fact cause any change to CE.

Imaging studies can also be interpreted as supporting the notion that M1 activity in response to motor movements might vary as a function of task novelty or learning status rather than the movement parameters of the task itself, for example, Puttemans et al. (2005) observed an increase in M1 activation during initial learning, but this subsided when automaticity was achieved. While imaging cortical activation is by no means an analogue of CE, it is possible that the corresponding decreases in activation and CE in terms of skill learning and performance improvement are epiphenomenal processes associated with a change in the nature of neural basis for the production of motor movements as learning occurs.

The fact that this study did not find an overt effect of handwriting on MEP amplitude or SICI does not preclude the possibility that the synaptic activity associated with handwriting might cause a metaplastic change to subsequent plasticity induction. In order to investigate the metaplastic potential of handwriting, it would be necessary to use quite a different research paradigm to that employed by the current study. Rather than using TMS to assess CE at baseline and then different time points post task, the protocol would most likely assess CE first after a handwriting task and then again following the use of a non-invasive brain stimulation (NIBS) technique. A change in CE following induction would be considered indicative of plasticity induction, and finding a difference between groups (or between sessions, in a within-subjects design) defined by the presence or absence of a handwriting task would be understood as an activity-dependent modulation of plasticity induction (or a metaplastic effect). Common techniques for inducing cortical plasticity include: paired

associative stimulation (PAS) which can either cause facilitation or depression depending on the inter-stimulus interval (PAS25 and PAS10, respectively); repetitive TMS (rTMS) which leads to facilitation at low frequencies (<1Hz) and depression at higher frequencies (>5Hz); theta burst stimulation, which induces facilitation when stimulation is intermittent (iTBS) and depression when continuous (cTBS) and finally transcranial direct current stimulation (tDCS) which varies in effect depending on the placement of anodal and cathodal electrodes on the scalp (Ridding & Ziemann, 2010).

The literature on the relationship between motor activity and plasticity induction at a systems-level is somewhat disparate to say the least. Motor activity is credited with causing a range of different effects, including suppression of the usual facilitatory effect of PAS25 but no change to the inhibitory effect of PAS10 (Stefan et al. 2006); reversal of the facilitatory effect of PAS25 to an effect of inhibition (Rosenkranz, Kacar & Rothwell, 2007); reversal of facilitatory effect of cTBS to inhibition (Gentner, et al., 2008); decrease in the extent of plasticity in the expected directions following iTBS and cTBS (Huang, 2008); reversal of the expected effects of iTBS and cTBS (Iezzi et al., 2008) and increased inter-subject variability in the effect of inhibition following cTBS (Goldworthy, 2014). There is also a raft of different effects observed when plasticity induction precedes motor activity (Takeuchi & Izumi, 2015) and in paradigms featuring different timings and combinations of NIBS (Ridding & Ziemann, 2010).

While it is likely that the large array of effects observed in relation to NIBS techniques reflects the complexity of the processes underpinning them, it also seems possible that, in the absence of a clear, overarching theory of metaplasticity in terms of a neurophysiological substrate, this field may be vulnerable to an over-reliance on significance-testing when interpreting data. Sometimes significant results occur randomly, and researchers should try to remain cognisant of the pitfalls of attempting to capitalise on

unexpected significant results with post facto interpretations declaring the presence of effects not previously considered or documented.

Sometimes non-significant results also occur randomly, and as such, another possible explanation of the current pattern of results would be that handwriting does affect CE, but that the current study was unable to detect this effect either due to a purely random fluctuation (which would most likely not occur again if the study were replicated) or perhaps due to some kind of a methodological error or mistake in data collection or experimental design. Given the exploratory nature of the current study, and the corresponding lack of literature in this area, the methodology employed could be construed as somewhat arbitrary at times. Although utmost effort was taken to ensure that there was an empirical basis for all techniques used, the novelty of this paradigm meant that in some instances, there was no option other than to base a parameter on previous research which may not have been valid in this context. For example, the five minute length of the handwriting task was based on timeframes used in the induction of changes to CE by fine motor tasks in previous research (e.g. Caramia et al., 2000, Garry, Kamen & Nordstrom, 2004; Rossi, Triggs & Eisenschenk, 1999). While fine motor activity is inarguably an important component of handwriting, when designing an exploratory research paradigm, it is difficult to infer the relative importance of other aspects of handwriting, such as its cognitive or linguistic demands, or status as an overlearned task, which might also have an influence on the time course of induction of neuroplastic changes.

Limitations

One possible limitation of the current study was the number of single and paired pulse MEPs evoked and measured at each time point and intensity. Although it is common to measure between 5 and 15 MEPs per condition (e.g. Karabanov et al. 2012; Pitcher, Ogston

& Miles, 2002; Ziemann et al., 2001), because of the high rates of inter and intra subject variability it might be advisable to measure more in order to increase reliability of estimates of CE. Research by Cuypers, Thijs and Meesen (2014) looking at the optimisation of single pulse TMS protocols in an inactive state suggests that measurement of at least 30 MEPs is required in order to have a 99% chance of landing within the 95% confidence interval of CE. However, as it takes time to deliver TMS and measure MEPs, increasing the number of MEPs measured might make measurement at certain time points post-task untenable, as the time taken to measure MEPs exceeds the interval between the designated time points. For example, the current study measured 10 MEPs per pulse type (paired and single) at each intensity plus a silent period at each time point (baseline, post 0 and post 15). This meant that at post 0, 80 MEPs were measured in total and this took around 10 minutes. Measuring three times as many MEPs would presumably cause the measurements taken at post 0 to run for more than 15 minutes and consequently some temporal resolution would be forgone. This is something of a catch-22 situation, where researchers must negotiate a trade-off between reliably measuring MEP amplitude and charting the time course of CE. Another possible option for decreasing variability in MEP amplitude might be to measure MEPs when the muscle of interest is slightly contracted. Darling, Wolf and Butler (2006) found that background muscle contractions of between 5-10% of maximum contraction caused a significant decrease in MEP variability.

An additional potential limitation of the current study may have been insufficient measurement intervals. For example, Caramia et al. (2000) found that under some circumstances, the time course of changes to CE can vary over a period of up to 30 minutes following task completion, with facilitation of MEP amplitude potentially only beginning at 15 minutes post task. Bueteifisch et al. (2000) also observed a continuation of task-related changes to MEP amplitude between 20 and 30 minutes post task. It is possible that the

decision to take ultimate MEP measurements at 15 minutes post-task might have prevented the current study from detecting effects which could plausibly have occurred after this point.

Implications and future research:

Given the dearth of previous research on overlearned tasks and cortical excitability, it seems clear that more research should be conducted in this field. The lack of a cohesive body of literature in this area makes interpretation of the absence of a main effect of time in the current study less straight forward, and thus directions for future research less specific. It is possible that the current study failed to detect an overt effect of handwriting because there was no effect to be detected but is also possible that the current study failed to detect an effect which was present, either randomly or because the current study paradigm did not have the requisite sensitivity to detect an effect. Conducting more research in this area and varying different parameters in terms of: length of handwriting task; timing of measurement post task; number of MEPs per condition and intensity of stimulation would undoubtedly elucidate the relationship between handwriting and overt effects on CE and would provide a context for the interpretation of the current study.

If the current study failed to detect an effect of handwriting on CE because handwriting cannot change CE, the implications of this would be effectively nil. There would be no reason to review research protocols in terms of controlling for handwriting behaviour prior to research participation, and any incidental handwriting involved in consent forms or medical screening should not be of concern. However, the design of the current study meant that it would only have been possible to detect an overt effect. If the current study did not detect an effect because indeed there was no overt effect, this would not necessarily have any bearing on the potential for handwriting tasks to cause a metaplastic effect.

The capacity of handwriting to influence metaplastic mechanisms remains uncharted water. As metaplastic effects have been observed following simple, tonic contractions of the hand muscles (Goldworthy et al. 2014), it seems plausible that a more complex task such as handwriting might also have the potential to influence plasticity induction. However, the design of the current study meant that any results pertaining to an overt effect of handwriting on CE would not have had any functional significance for metaplastic research; the only implications the current study could have had for research in this direction is the possibility of piquing interest in metaplastic effects of overlearned tasks more generally.

Conclusions

The basis of the rationale for this study was the dearth of literature on overlearned tasks and cortical excitability combined with the potential for major methodological implications were an effect to be detected. Given the exploratory nature of this study, the hypotheses were somewhat arbitrary and ultimately were not supported by observation as there was no clear emergence of an effect of time on MEP amplitude or SICI as indices of CE. It remains unclear as to whether this indicates an absence of effect, or the scope of this particular study was not sufficient to detect an effect. The trending towards significance of the main effect of time on MEP amplitude might provide tentative support for the presence of an effect of handwriting on CE, but the results are far from conclusive. Further investigation in this area, perhaps varying timing and task parameters would shed light on the findings of the current study. As the absence of overt effects such as those which might have been detected in the present study has little physiological bearing on whether or not handwriting or other overlearned everyday tasks might influence subsequent plasticity induction, there is no reason for this null result to stifle further research or cast doubt over the broader rationale. The

current finding can be seen to represent a small and inconclusive piece of a larger puzzle which continues to be of relevance in the area of TMS research.

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Appendix B

Information Sheet

The effect of hand writing on cortical excitability

Information sheet for study participants

1. Invitation

You are invited to participate in a study investigating the effect of hand writing on the brain systems that control hand movements. The aim of the research is to improve our understanding of how well-learned tasks that are performed during our daily lives affect the neural systems that support the learning of novel tasks.

The study is being conducted by:

- Dr Mike Garry, School of Medicine (Psychology), University of Tasmania
- Ms Lillian Brinken (Honours student), School of Medicine (Psychology), University of Tasmania
- Ms Mona Thorpe (Honours student), School of Medicine (Psychology), University of Tasmania

This study is being conducted in partial fulfillment of Honours degrees for Lillian Brinken and Mona Thorpe under the supervision of Dr Mike Garry. The study will be take place in the Human Motor Control laboratory, Psychology Research Centre, University of Tasmania, (03) 2662 2204.

2. What is the purpose of this study?

The study is being conducted to improve our understanding of how the brain and nervous system are affected by the performance of common, everyday tasks. The specific focus of this study is whether, and how, handwriting tasks influence the parts of the brain and nervous system that control movement.

The findings from this study will help to improve understanding of how the brain and nervous system control movements. This knowledge will help with the development and refinement of rehabilitation therapies for people that have suffered brain injuries such as stroke.

3. Why have I been invited to participate?

As you are between 18 and 45 years of age, are right-handed, and have normal or corrected-to-normal vision you have been invited to participate in this research. We want to emphasise that your participation is voluntary and that you are free to withdraw at any time.

The technique of transcranial magnetic stimulation (TMS) used in this study is very safe, but there are certain conditions that will exclude some people from participating. You will be asked to complete a medical screening questionnaire to ensure that you are free of any exclusionary criteria.

Exclusion criteria include:

- epilepsy, or a family history of epilepsy
- history of unexplained seizures (fits)
- serious head injury (e.g., concussion) requiring hospitalisation within the last three years
- implanted electronic devices such as pacemakers
- metal implants or metal fragments in the head (excluding dental work)
- history of migraines
- pregnancy

Certain medications (for example some types of anti-depressant medications) can influence how the brain responds to sensory stimulation and voluntary movements. Therefore, we ask that you inform the experimenter if you are taking any medication prior to participating in the study.

4. What will I be asked to do?

This study will involve you completing two (2) separate testing sessions, each lasting approximately 90 minutes, at least seven (7) days apart. These will be scheduled at times that are convenient for you. Prior to the first session you will be asked to complete a short, questionnaire to collect demographic information (age, sex, etc.), assess handedness and screen for exclusion criteria for transcranial magnetic stimulation (TMS). If you are free of all exclusion criteria you continue to the main part of the study.

At the beginning of each session sticky recording electrodes will be placed on the skin over two muscles of your right hand: one muscle that moves your index finger, and one muscle that moves your thumb. To ensure the best possible recording of the activity of these muscles, the skin will be prepared by scrubbing it with a mildly abrasive paste and then cleaning it with an alcohol wipe. If there is hair on the skin a small area will be shaved using a disposable razor. This procedure may produce some minor irritation of the skin (e.g., redness). The adhesives used on the electrodes are hypoallergenic. Wires will then be connected to the electrodes so a recording device (EMG system) can record muscle activity during the experiment.

The technique of transcranial magnetic stimulation (TMS) will be used to stimulate the area of the brain that controls muscles of the right hand. TMS is a safe, painless technique used to measure changes in the activity of the brain during the study. Electromagnetic ‘pulses’ will be delivered through a coil held against your scalp by the investigator. To ensure the coil is always positioned in the same place, a felt-tip pen will be used to mark the location on your scalp. This mark will be removed at the end of the session using an alcohol wipe. When a TMS pulse is delivered you will hear ‘click’ sound from the coil and muscles of the hand/arm will ‘twitch’. You may also feel a ‘tap’ sensation on your scalp and muscles around the eye may twitch, causing the eye to blink. This may feel a bit strange but it is not painful.

TMS will be used to measure brain activity at five times during the study. Each of these ‘blocks’ of TMS stimulation will take approximately nine (9) minutes to complete, and there will be approximately six minutes between blocks. Approximately 100 TMS pulses will be delivered in each block. For the majority of the block you will be asked to sit quietly with your hand muscles relaxed, but for approximately one minute of each block you will be asked to lightly grip a pen held between the index finger and thumb of your right hand.

During the interval between the second and third TMS block you will be asked to perform simple handwriting task. This task will differ in the two sessions. In one session you will be asked to copy of 120 word passage of text onto a piece of paper. In the other session you will be asked to repeatedly draw a set of three geometric symbols: triangle, circle and square. in total you will draw this set of symbols 200 times. Following the drawing task, the remaining three blocks of TMS will be given.

After the final TMS block, the electrodes will be removed from your hand and you will be free to leave.

5. Are there any possible benefits from participation in this study?

Your involvement in this study will aid in the understanding of the brain and nervous system’s role in the control of movement. The findings from the study will contribute to the development of techniques to improve recovery of function following brain injury, such as stroke.

First-year psychology students will receive 3 hours course credit following completion of both sessions (i.e., 1.5 hours for each session). If you are not a first year student, or have already received full participation credit, you will be entered into a draw to receive one of two \$50 Coles-Meyer gift vouchers.

6. Are there any possible risks from participation in this study?

There are few risks associated with the procedures used in this study. The TMS pulse may cause muscles of the scalp to 'twitch' (e.g., can cause the eye to blink). This may feel 'odd', but is not painful. On rare occasions TMS can cause a 'muscle tension' type headache. TMS requires self-adhesive electrodes to be placed on the skin. The skin will need to be prepared prior to application of these electrodes. This will involve scrubbing the skin with a mildly abrasive paste and shaving the skin using a disposable razor to remove any hair. These may cause some mild skin irritation and redness.

Some people experience 'vasovagal syncope', or fainting, in response to certain 'trigger' stimuli. Common triggers for sensitive individuals include health-related procedures, such as needles or the sight of blood, and stress and anxiety. For a small percentage of people, TMS can trigger a fainting reaction. If you have experienced fainting previously, please let us know.

7. What if I change my mind during or after the study?

It is important that you understand that your participation in this study is completely voluntary and you are free to withdraw at any time without prejudice. If you decide not to participate you may do so without providing an explanation. You will be asked to sign a Statement of Informed Consent to indicate your full understanding of the purpose and requirements of your participation. However, if you find that you are becoming distressed, we will arrange for you to see a University counselor at no expense to you. Should you choose to withdraw from the study, any information provided during your participation will, if possible, be excluded from the study.

8. What will happen to the information when this study is over?

After this study has been completed, all data will be kept for five years. Electronic documents will be stored on a password protected computer in the Human Movement and Neuroscience Laboratory at the University of Tasmania, Hobart Campus. All other documents will be stored in locked filing cabinets on the Hobart Campus. All information will be treated in a confidential manner, and your name will not be used in any publication arising out of the research. This data can only be accessed by the Chief Investigator and Student researcher. After a five year duration the data will be destroyed by deletion of electronic documents and shredding of other documents.

9. How will the results of the study be published?

The results of this study will be disseminated in a research thesis, as well as in a presentation to fellow Honours students and their supervisors. The study results will also be submitted for publication in a peer-reviewed, neuroscience research journal. Participants will not be identifiable in the publication of results.

10. What if I have questions about this study?

If you would like to discuss any aspect of this study please feel free to contact either Mike Garry on (03) 6226 2204, Lillian Brinken (lbrinken@utas.edu.au), or Mona Thorpe (mthorpe0@utas.edu.au). Any of us would be happy to discuss any aspect of the research

with you. Once we have analyzed the information we will be mailing / emailing you a summary of our findings. You are welcome to contact us at that time to discuss any issue relating to the research study.

This study has been approved by the Tasmanian Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, please contact the Executive Officer of the HREC (Tasmania) Network on +61 3 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. Please quote ethics reference number [H0009261].

Thank you for taking the time to consider this study. If you wish to take part in it, please sign the attached consent form. This information sheet is for you to keep.

Appendix C

Consent Form

The effect of hand writing on cortical excitability

This consent form is for research participants.

1. I agree to take part in the research study named above.
2. I have read and understood the Information Sheet for this study.
3. The nature and possible effects of the study have been explained to me.
4. I understand that the study involves two sessions of approximately 90 minutes each, at least seven days apart. In each session, sticky electrodes will be placed on my right hand to allow recording of muscle activity, and transcranial magnetic stimulation will be used to measure brain activity. I will perform a short handwriting task (approximately five minutes) in each session.
5. I understand that participation involves the risk(s) that skin preparation for muscle recording may cause mild discomfort and that transcranial magnetic stimulation will produce a click sound and muscle twitches of the face and hand. I will complete a medical screening questionnaire to ensure I am free of exclusion criteria for transcranial magnetic stimulation.
6. I understand that all research data will be securely stored on the University of Tasmania, Sandy Campus premises for five years from the publication of the study results, and will then be destroyed unless I give permission for my data to be stored in an archive.

I agree to have my study data archived.

Yes ☐ No ☐

7. Any questions that I have asked have been answered to my satisfaction.
8. I understand that the researcher(s) will maintain confidentiality and that any information I supply to the researcher(s) will be used only for the purposes of the research.
9. I understand that the results of the study will be published so that I cannot be identified as a participant.
10. I understand that my participation is voluntary and that I may withdraw at any time without any effect.

If I so wish, I may request that any data I have supplied be withdrawn from the research until August 31, 2014 after which the data will be included in the Honours theses of Mona Thorpe and Lillian Brinken.

Participant's name: _____

Participant's signature: _____

Date: _____

Statement by Investigator

☐ I have explained the project and the implications of participation in it to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation.

If the Investigator has not had an opportunity to talk to participants prior to them participating, the following must be ticked.

☐ The participant has received the Information Sheet where my details have been provided so participants have had the opportunity to contact me prior to consenting to participate in this project.

Investigator's name: _____

Investigator's signature: _____

Date: _____

Appendix D

Medical Screening and Handedness form

Medical History and Handedness

Participant Code..... Age..... Sex: M / F

Exclusion criteria

Do any of the following apply to you?

- | | | |
|--|-----|----|
| • epilepsy, or a family history of epilepsy | yes | no |
| • history of unexplained seizures (fits) | yes | no |
| • serious head injury (e.g., concussion) that required hospitalisation with the last three years | yes | no |
| • implanted electronic devices such as pacemakers | yes | no |
| • metal implants or metal fragments in the head (excluding dental work) | yes | no |
| • history of migraines | yes | no |
| • currently pregnant or could be pregnant | yes | no |

Medical History

Are you currently suffering from anxiety or depression?.....

Do you have a heart condition or any other serious physical condition?

.....

Are you currently taking any prescription medication? If so, what medication?

.....

Have in the past taken any medications for psychological condition(s)? If so, what medications?

.....

Have you ever had or are you now suffering from any of the following (please circle):

Stroke	Yes	No
High Blood Pressure > 140 / 90	Yes	No
Diabetes	Yes	No
Arthritis	Yes	No
Fits or convulsions	Yes	No
Epilepsy	Yes	No
Giddiness	Yes	No
Concussion	Yes	No
Severe Head Injury	Yes	No
Loss of Consciousness	Yes	No

Handedness

For each of the activities below, please tell us:

1. Which hand do you prefer for that activity?
2. Do you *ever* use the other hand for the activity?

	Preferred hand?		Ever use other hand?	
Writing	L	R	Y	N
Drawing	L	R	Y	N
Throwing	L	R	Y	N
Using scissors	L	R	Y	N
Using a toothbrush	L	R	Y	N
Using a knife (without fork)	L	R	Y	N
Using a spoon	L	R	Y	N
Using a broom (upper hand)	L	R	Y	N
Striking a match	L	R	Y	N
Opening a box (lid)	L	R	Y	N

Do you ever confuse left and right?

How many people in your immediate family are left handed?

Thank you.

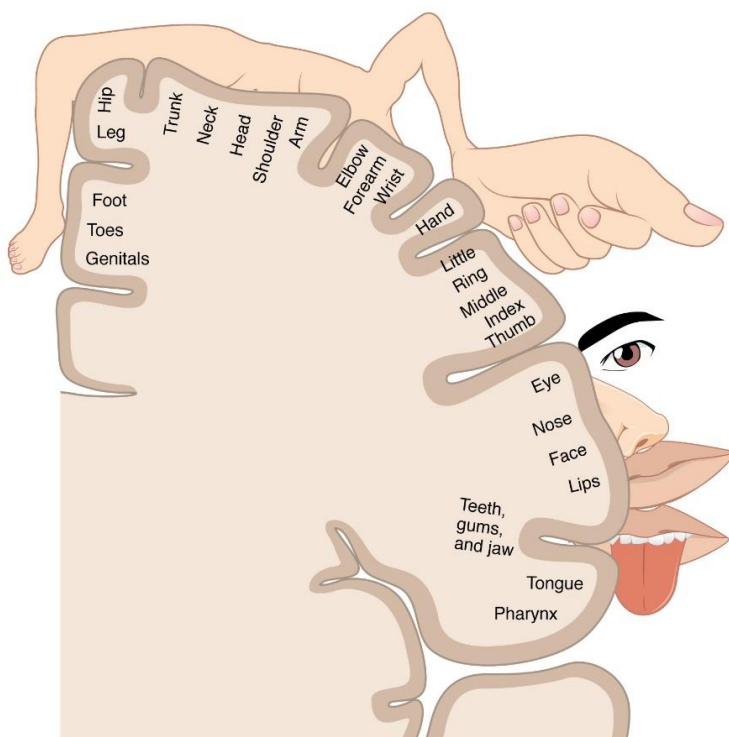
Appendix E

Advertisement for participant recruitment

Do you love science?

How much? Enough to **help science** if science needed you? Here is your chance to show how much you love science by participating in an exciting, non-invasive study into **Neuroplasticity** and every-day tasks. Eligible participants are right-handed and aged between 18-45. Course credit available for first year Psychology students, and other participants go into a draw to win gift vouchers.

Email Lily, lbrinken@utas.edu.au or Mona mthorpe0@utas.edu.au for more information.



Appendix F

Text Used for Writing Task

Text writing task

When we conduct a chi-square test of independence, we're comparing groups in terms of some outcome variable. For example, you might be comparing two groups of students (those who ate breakfast and those who didn't) in terms of propensity to fall asleep in class. One very important thing to understand here is that with a chi-square test, we're comparing groups in terms of the *proportion* or *percentage* obtained on the outcome variable. In the sleeping-in-class example, we would be comparing our two groups in terms of the percentage (or proportion) of students who fell asleep in class. Was the percentage of students who fell asleep in class higher in the group who skipped breakfast than the group who ate breakfast?

Appendix G

Multivariate Data

Table G1

ANOVA for MEPs at FDI

Multivariate	<i>Df</i>	<i>F</i>	ηp^2	<i>P</i>
Time	2	3.51	.334	.058
Error (Time)	14			
TMS type	1	23.88	.614	.000*
Error (TMS type)	15			
Intensity	2	11.69	.625	.001*
Error (Intensity)	14			
Time x TMS type	2	0.74	.096	.495
Error (Time x TMS type)	14			
Time x Intensity	4	0.77	.203	.568
Error (Time x Intensity)	12			
TMS type x Intensity	2	5.22	.427	.020*
Error (TMS type x Intensity)	14			
Time x TMS type x Intensity	4	0.19	.060	.938
Error (TMS type x Intensity)	12			

Note: Asterisks denote significant *p* values.

Table G2

ANOVA of MEP amplitude at APB

Multivariate	<i>df</i>	<i>F</i>	ηp^2	<i>p</i>
Time	2	3.13	.309	.075
Error (Time)	14			
TMS type	1	15.96	.515	.001*
Error (TMS type)	15			
Intensity	2	13.30	.655	.001*
Error (Intensity)	14			
Time x TMS type	2	0.90	.114	.430
Error (Time x TMS type)	14			
Time x Intensity	4	1.47	.328	.272
Error (Time x Intensity)	12			
TMS type x intensity	2	1.61	.186	.236
Error (TMS type x intensity)	14			
Time x TMS type x Intensity	4	0.38	.113	.816
Erro (Time x TMS type x Intensity)	12			

Note: Asterisks denote significant *p* values.

Table G3

ANOVA for LOG SICI at FDI

Multivariate	<i>Df</i>	<i>F</i>	ηp^2	<i>P</i>
Time	2	0.60	.079	.561.
Error (Time)	14			
Intensity	2	3.49	.333	.059
Error (Intensity)	14			
Time x Intensity	4	0.07	.024	.989
Error (Time x Intensity)	12			

Note: Asterisks denote significant *p* values.

Table G4

ANOVA for LOG SICI at APB

Multivariate	<i>Df</i>	<i>F</i>	ηp^2	<i>P</i>
Time	2	1.26	.153	.313
Error (Time)	14			
Intensity	2	1.84	.208	.195
Error (Intensity)	14			
Time x Intensity	4	0.76	.202	.570
Error (Time x Intensity)	12			

Note: Asterisks denote significant *p* values.

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HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

12 June 2014

Dr Michael Garry
Psychology
Private Bag 30

Sent via email

Dear Dr Garry

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT
Ethics Ref: **H0009261 - Bilateral movement therapy in post-stroke hemiparesis**

- Change to investigators: addition of Honours students Ms Lillian Brinken and Ms Mona Thorpe, removal of Ms Monica Lovell.
- Change of task participants perform to a brief handwriting task.
- Narrowing of age range from 18-50 to 18-45.
- Revised Information Sheet and Consent Form to reflect changes to procedures and relocation of Psychology to the Faculty of Health.

We are pleased to advise that the Chair of the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 11 June 2014.

Yours sincerely

Katherine Shaw
Executive Officer
Tasmania Social Sciences HREC